2016 Protocols

Illumina MiSeq 16S rRNA Sequencing Analysis

Adapted from:
16S standard operating procedure as outlined in
https://github.com/mlangill/microbiome_helper/wiki/16S-standard-operating-procedure
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Purpose:
To perform compositional analysis on Illumina MiSeq 16S amplicon datasets.

Requirements:
- Command line terminal (e.g. Mac OS Terminal, VirtualBox)
- FastQC
- PEAR
- FASTX toolkit (v0.0.14)
- VSEARCH
- QIIME
- SortMeRNA
- SUMACLUST
- PICRUSt
- STAMP

Procedure:
(Reference: 16S standard operating procedure as outlined in https://github.com/mlangill/microbiome_helper/wiki/16S-standard-operating-procedure: terminal commands also found here.)

1. (Optional) Run FastQC to allow manual inspection of the quality of sequences.
2. Stitch paired end reads together.
3. Filter stitched reads by quality score, length and ensure forward and reverse primers match each read.
4. Convert FASTQ stitched files to FASTA and remove any sequences that have an 'N' in them.
5. Remove chimeric sequences with VSEARCH.
6. Create a QIIME "map.txt" file with the first column containing the sample names and another column called "FileInput" containing the filenames. This is a tab-delimited file and there must be columns named "BarcodeSequence" and "LinkerPrimerSequence" that are empty.
7. Combine files into single QIIME "seqs.fna" file.
8. Create OUT picking parameter file.
9. Run entire QIIME open reference picking pipeline with the new sortmerna and sumaclust. You may want to change the subsampling percentage to a higher amount from the default.
10. Filter OUT table to remove singletons as well as low-confidence OTUs that are likely due to MiSeq bleed-through between runs.
11. Summarize OTU table to determine number of sequences per sample.
12. Normalize OTU table to sample sample depth.
13. Manually add column(s) to map.txt that contain information to group your samples (e.g. healthy vs. disease).
14. Create UniFrace beta diversity plots.
15. Create alpha diversity rarefaction plot.

16. Convert BIOM OTU table to tab-separated file to be opened/explored in text editors or Excel, etc.
17. Convert BIOM OTU table to STAMP.